

THE EFFECT OF PECTIN AND CELLULOSE  
ON PROTEIN UTILIZATION  
IN OLDER ADULT RATS

BY

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## INTRODUCTION

The role of dietary fiber in animal nutrition is of increasing interest, as its physical, chemical and metabolic effects on the gastrointestinal tract are reported. Although fiber is largely unsusceptible to the digestive processes in monogastric animals, possible effects on other substances in the gut are important. The consumption of a highly refined diet, low in dietary fiber, has been implicated in the causation of various disorders such as atherosclerosis, colon and rectum cancer, diverticulosis, ischemic heart disease, kidney stones and constipation (1-7). Therefore, increasing the level of fiber in the diet has been suggested for its possible therapeutic value (3). Some investigators have found that higher levels of fiber in the diet have adverse effects on nutrient absorption, especially minerals (8,9). The effects of some fiber sources on nitrogen utilization from the diet have also been investigated in which nitrogen excretion was often increased, but effects on nitrogen equilibrium and utilization were varied (47, 48).

Before increases in the consumption of dietary fiber are recommended in the fields of nutrition and medicine, investigations are needed concerning the possible deleterious effects high levels of fiber may have on other nutrients. Special emphasis is needed on older animals in this research, as the middle-aged, and particularly the elderly populations are more inclined to develop the above disorders.

Protein utilization and requirements in the aged organism are under current debate. Differences in cell and tissue function, when aged and young animals were compared, have been found (10-18). Decreased rate of protein synthesis (13-15) and increased protein requirements (16-17) in older animals have been reported. Concern about the adequacy of protein intake by elderly humans has been expressed (18) and general malnutrition or undernutrition in this population group have been recognized (19). Before increased levels of dietary fiber can be recommended for the diets of older persons, in an effort to combat some disorder, its possible effect on protein utilization should be considered.

This research was conducted to determine the effect of moderate levels of pectin and cellulose in the diet on protein utilization in older rats.

## REVIEW OF LITERATURE

### Protein Metabolism and Requirements in Older Animals

Changes in body composition and levels of blood proteins have been found in old animals when compared to young animals. Serum albumins decreased and serum globulins increased with advancing age (18). Carcass nitrogen was 14% lower and formed creatinine was 23% lower in aged mice when one and two year old animals were compared (20). The lowered total carcass nitrogen has been attributed to muscle loss in aging mice (10). Elderly women, ages 67-91, exhibited decreased body cell mass determined by counting the naturally occurring isotope  $^{40}\text{K}$  in the whole body (12). Shock reported that aging was characterized by a loss of tissue and functioning cells which may be the result of inadequate cellular nutrition (11).

This loss of functioning tissue in aging could lead to the reduction in reserve capacities and the ability to handle stress that has been noted (11, 15). When working with isolated mouse hearts from 1 to 3 month, 8 to 9 month, and 25 to 27 month old animals, Geary and Florini (15) found protein synthesis, as measured by labeled leucine incorporation into protein, reached a maximum level in adult mouse hearts and decreased substantially with greater age. They concluded that the hearts of senescent animals were less capable of compensating for stress by increasing the amounts or kinds of protein formed. Wannemacher and McCoy (17) studied young and old dogs, to determine the optimal nitrogen intake for filling of the protein reserves, as

measured by liver and muscle biopsies and serum proteins. Experiments were conducted on a depletion, repletion regime with varying amounts of nitrogen in the diets. Older dogs were found to have a higher daily requirement for nitrogen per kilogram of body weight, to obtain maximal protein reserves than younger dogs (17). The authors noted that adequate reserves were necessary for animals to respond optimally to stress. The older dogs also had a less efficient, slower rate of anabolism when compared to the younger dogs (17).

Decreased rate of protein synthesis in older animals has been noted by other researchers (10, 13, 14, 21, 22) in studies involving various body compartments. Short found a slower rate of amino acid incorporation into muscle protein in 98 to 113 day old mice than in 71 to 79 day old animals (13). This experiment used animals that were much younger than those normally used in aging research. The free and total amino acid concentration in tissues and in blood were compared, from 2 to 3 month old and 18 to 24 month old animals, by Oeiru (14). The aged rats were found to have greater concentrations of free amino acids, yet smaller total amino acid concentration in serum and tissues suggesting lower intensity of protein biosynthesis. Mainwaring (21) found that livers from 30 month old mice were less capable of synthesizing protein than livers from 5 month old animals. He suggested that the microsomes in the old tissue were less responsive to synthetic mRNA, and old age may be accompanied by less mRNA in cells.

Sobel and Bowman (10) found that the reduced rate of protein synthesis with aging was accompanied by a reduced rate of protein

breakdown. The protein content of the carcass was significantly lower in 320 to 750 day old mice, when compared with 3 month old controls. However, the half life of isotopically labeled protein increased progressively in the older animals. The authors contended that this slower breakdown of labeled protein could be caused by a slower turnover rate, a breakdown of old protein before newly-synthesized protein or a more efficient reutilization of protein in old animals. However, the decrease in protein synthesis was greater than the slowed breakdown, thus causing the loss in total carcass protein with age. When newborn infants, young adults and elderly females were compared by Young et al. (22), the rate of protein synthesis was found to decrease continually throughout the life span. However this decline was eliminated when compared in terms of basal energy metabolism.

A study of obligatory nitrogen losses in elderly women suggested there was increased turnover of body protein with age, since urinary nitrogen losses per unit of body cell mass were higher for older than younger women (12). This finding conflicted with the report of Sobel and Bowman from their mouse study. The higher urinary nitrogen losses could also have been caused by a reduced ability to conserve nitrogen in the aged women on a protein-free diet. The obligatory fecal nitrogen losses were similar for both age groups. Computation of the protein requirement for older women from obligatory loss data by the authors was lower than the level reported for maintenance of nitrogen balance in the elderly. The authors hypothesized that there may be less efficient metabolism of ingested protein in older persons.

Tuttle et al. (16) investigated the requirement for essential amino acid nitrogen in 50 to 70 year old men. Five of the six elderly subjects required greater essential amino acid nitrogen to attain nitrogen balance than younger men did. Therefore, the authors concluded that there may be a higher requirement for essential amino acid nitrogen in older men.

Watkin (18) reported on the nitrogen balance of elderly and middle-aged men fed different levels of protein. The two groups showed equal ability to adapt to the dietary changes of medium, low, and high protein regimes. The author concluded that the rate of protein synthesis in the elderly would increase as soon as the needed protein substrate was available from the diet. Amino acid studies which found increased requirement for essential amino acids, especially methionine and lysine, in the elderly were reviewed (18).

Watkin (18) suggested that the great variability in aging research results was partly due to the variety of factors that effect the two parameters commonly used, nitrogen balance and serum albumin concentrations. Long experiments with tightly controlled observations were recommended to look fully at nutrition and aging. The older animal seems to have less efficient metabolism and slower synthesis of protein. Dietary requirements may be greater in the aged, particularly essential amino acid needs. It would be helpful to examine the interaction of ingested protein in the older adult rat to other dietary components, such as fiber.

### Metabolic Effects of Dietary Fiber, Pectin and Cellulose

The concept of a fiber deficiency in the diet of "Westernized" countries originated in part from epidemiological data, comparing populations of the United States or Great Britain, with rural African inhabitants (5, 6, 23). In general, the African population had larger daily stools, decreased transit times, and a low incidence of diverticulosis, appendicitis, colon and rectum cancer and atherosclerosis, accompanied by their consumption of a diet high in natural fiber, low in refined foods. Experiments have been conducted in animals and humans to determine the validity of these arguments. Dietary fiber is proposed to decrease plasma lipid levels, decrease intestinal transit time, increase excretion of bile acids, and reduce concentration of sterols and other components in the feces. Literature reports do not support all these claims however, for all dietary fiber sources. For the purposes of this study, reports dealing mainly with the purified dietary fiber sources of pectin and cellulose will be cited.

The hypocholesterolemic effect of pectin has been reported in man (24,25), and in rats (26-29). Adult males, 21 to 32 years old, were given 36 g pectin per day for periods of two weeks, alternating with periods of no fiber supplements (24). Serum cholesterol was significantly lowered with pectin but rose back to the original levels when supplementation was discontinued. There was an increased loss of fat in the feces with pectin supplementation. The authors suggested that increased bile acid loss may have occurred with the steatorrhea, causing a decrease in serum cholesterol. In an experiment with 30 to

55 year old men, pectin also exhibited a hypocholesterolemic effect (25).

Wells and Ershoff (26) found that pectin, fed as 2.5%, 5%, or 10% of the diet, linearly lowered serum and liver cholesterol in young rats fed a cholesterol-supplemented diet. Leveille and Säuberlich (27) found that rats fed a 5% pectin diet, with 1% cholesterol, had reduced serum and liver cholesterol and greater bile acid and cholesterol excretion in the feces. An in vitro study by these authors showed that pectin inhibited the reabsorption of bile acids. It was concluded that the hypocholesterolemic effect of pectin was caused by its effects on bile acid and cholesterol absorption. The authors noted that pectin did not appear to interfere with the absorption of other nutrients since there was no change in body weight gain in the pectin-fed rats.

This finding was not supported by Mokady (28) who studied the metabolic effects of various types of pectin in weanling rats. In his experiments, 10% pectin in the diet retarded growth slightly, reduced serum cholesterol, and increased fecal total lipids and fecal sterols. The growth retardation may have been caused by pectin's interaction with other nutrients such as protein in the gut. His experimental diets contained 10% casein, compared to 18% casein in the study of Leveille and Sauberlich (27). Therefore, if pectin did affect protein absorption, the animals in Mokady's experiment would have been less likely to meet their dietary requirement. High methoxy, high viscosity pectin was found to have the greatest hypocholesterolemic effect. Mokady (28) hypothesized that the pectin surrounded fat

globules in the intestine, interfering with emulsification, or that pectin adsorbed to the walls of the intestine, thus causing decreased lipid absorption. Tsai et al. (29) found a lowering of serum cholesterol in two out of three experiments with young rats, given diets with 7% pectin and 0.5% cholesterol. Liver cholesterol was also reduced. The lack of hypocholesterolemic effect in the third experiment by pectin was attributed to the greater fat component of the diet, which was 15%, compared to 10% in the diets where an effect was shown. It was also found that pectin increased total body cholesterol, apparently causing a movement of the sterol from the liver and serum pools into the tissues.

In an experiment with two year old cockrels fed diets with 5% pectin or 5% cellulose for 18 months, the pectin-fed birds had fewer atherosclerotic plaques in the aorta than the control, cellulose-fed birds (30). Yet the pectin produced significantly higher serum cholesterol levels and apparently reduced overall nutrient utilization, with the control birds gaining as much as three times more weight. The authors suggested that pectin would still be helpful in depressing avian atherosclerosis, probably by accelerating the rate of food passage through the gut. Interference with the absorption or reabsorption of atherogenic compounds, such as cholesterol, was questioned, however, due to the higher serum cholesterol values. An experiment with young rats fed a 5% pectin diet for 14 days also failed to show decreased cholesterol in the plasma, but total fecal steroid excretion did increase (31).

The metabolic effects of a 10% or 20% cellulose diet were ex-

tensively investigated by Morgan et al. (32) in an experiment with young rats. Animals were maintained on a fiber free diet for 10 days, than randomly assigned by weight, to treatments varying in the level and source of fiber. Experimental feeding periods were 14 or 28 days. Fiber diets led to a significant increase in fecal output, largely due to an increase in solids. The level of bile acids in the feces, was greater for cellulose-fed rats than control rats while bagasse-fed animals had significantly greater loss of bile acids than either group. The animals fed 20% cellulose had reduced efficiency of feed utilization, i.e. gained less weight per gram of food eaten. Serum cholesterol levels were not changed by the feeding of 10% or 20% cellulose.

The feeding of a high cellulose ration has been found to decrease serum cholesterol levels, but only when cholesterol has been added to the diet and when the animals were obese. Sunderavalli et al. (33) found liver and serum cholesterol were significantly lower with a 20% cellulose and 1.5% cholesterol supplemented diet than with the same diet without cellulose. In this five week experiment with weanling rats the cellulose-fed animals also excreted significantly more bile acids in the feces than did control rats. The authors suggested that this increased excretion was responsible for the decreased cholesterol levels. After producing obesity in young rats in another experiment, with a 60% fat diet, Sunderavalli and co-workers (34) fed restricted diets to two groups of rats, six g per day, and supplemented one group with an additional two g of cellulose. The animals fed the cellulose-supplemented diet lost more weight during the six week period than the

control group. All rats maintained a slightly positive nitrogen balance despite the low calorie intake. Following weight reduction diets, animals had significantly lower serum and liver lipids and serum and liver cholesterol than the obese rats that were sacrificed. The cellulose-fed group had slightly less serum cholesterol and significantly less serum lipids than the restricted diet without cellulose. The authors restated their previous suggestion that this cholesterol lowering effect may be due to increased fecal loss of bile acids.

The findings of the latter two investigations were not supported by other studies with rats or humans (2, 29, 35). Eastwood et al. (2) conducted a study with 12 men, 25 to 43 years old, who consumed a regular diet with additions of 16 g of bran, or 16 g of cellulose daily for a three week period. Both fiber sources produced similar results. There were marked increases in fecal weights, but no change in transit time, total bile acids excreted or serum cholesterol. The concentration of bile acids in the stool, however, was significantly reduced. The findings of Tsai et al. (29) supported these observations. A 7% cellulose diet fed for 42 or 32 days to young rats had no significant effect on serum or liver cholesterol, in comparison to control animals fed a no-fiber diet. Ahrens and co-workers (35) fed three groups of 300 day old rats a basal diet, the basal diet supplemented with cake or a basal diet supplemented with cake containing 9.5% cellulose. The cellulose-fed animals had significantly greater carcass cholesterol and slightly greater serum cholesterol than the other two groups. The authors viewed these findings as a possible threat to good health.

Many investigators have fed cellulose to experimental groups to compare its metabolic effects with other dietary fibers. A high level of cellulose supplementation seems to be required for the exhibition of any hypocholesterolemic effect.

#### Metabolic Interactions of Dietary Fiber with Protein

The effects of high fiber diets on dietary protein has been examined in animals and in man. Early research conducted with humans by Morgan (36) found that 20 g of cellulose per day increased fecal dry weight and the amount of nitrogen lost in the feces. All subjects, however, remained in positive nitrogen balance. The human subjects of Adolph and Wu (37) also maintained a positive nitrogen balance when fed 12 g or 24 g of crude fiber with an experimental meat-rice diet. The apparent digestibility of the protein in the diet, however, decreased with the larger fiber supplement. The authors suggested that the lowered digestibility was caused by the faster passage of food material through the intestine, thus decreasing the time for absorption.

Lloyd and Cramer (38), feeding malt sprouts, wheat bran, linseed oil meal or oat groats to swine, reported that successive increases in crude fiber, from 1.4 to 8.6% of the diet, caused decreased protein digestibility. Rabbits fed a 4.5% or 11.1% crude fiber diet, also showed significantly lowered apparent protein digestibility and greater fecal nitrogen losses with the high fiber diet (39). In growing pigs however, wheat bran fed as 4.0 to 11.0% crude fiber, did not alter protein digestibility even though the animals showed decreased nitrogen

retention, food intake and weight gain with the high fiber diet (40).

Fecal nitrogen loss has been found to increase with high levels of cellulose feeding in pigs and rats. Cunningham et al. found that diets with 23.2% or 40% solka-floc, a purified source of cellulose, had a negative effect on growth in pigs (41) and significantly decreased nitrogen retention during the early growing period (42). Whiting and Bezeau (43) found that increased metabolic fecal nitrogen was largely responsible for the increased fecal nitrogen losses with high fiber feeding in swine. Their experiment showed that the greater the cellulose component of the diet the lower the true digestibility of the protein. They hypothesized that the cellulose could interfere with protein absorption and that the protein requirement for pigs would be higher with rations high in fiber. Meyer (44) reported similar results with rats fed a 0% or 30% cellulose diet for 28 days. He concluded that rats fed a 30% cellulose diet had a 1.85% additional need for dietary crude casein to meet protein requirements.

In man, higher fiber levels have consistently produced greater fecal nitrogen losses (7, 8, 45, 46). Walker (7) conducted an experiment with 9-12 year old South African children in which he added fiber supplements to the normal diet. When 4 g of fiber was added in the form of oranges the concentration of nitrogen and fat in the feces was lowered by the significantly greater total fecal excretion. He hypothesized that the increased total amount of nitrogen lost in the feces was from endogenous sources and that dietary protein was fully absorbed and digested. Reinhold et al. (8) did not support

this hypothesis with his findings from research on consumption of a high fiber bread by man. The increased fecal nitrogen excretion by his subjects was attributed to the lowered digestibility of the high fiber wheat bread. Despite the greater nitrogen fecal losses, there was no real change in nitrogen equilibrium, total serum protein or serum albumin.

The effects of 17 g vs 45 g of wheat fiber per day for three weeks on fecal nitrogen losses of six 21 to 25 year old males was reported by Cummings et al. (45). Fecal nitrogen losses increased significantly from 12 g per day to 20 g per day with the high fiber diet. This loss was not viewed as nutritionally significant by the authors, but was emphasized as an effect of the fiber on digestive functions in the small intestine.

Southgate and Durnin (46) reported fecal nitrogen losses in young and old males and females when consuming diets with varying levels of unavailable carbohydrate for two week periods. Significantly greater nitrogen was lost in the feces with greater unavailable carbohydrate in the diet. All subjects, however, remained in positive nitrogen balance with the elderly in a more positive state than the younger subjects. The apparent digestibility of the protein in the diet with greater fiber was significantly reduced for all age and sex groups. The authors suggested that the increased fiber accelerated the passage of the food through the gut, thus decreasing absorption and causing the greater fecal nitrogen losses.

The effect of pectin and cellulose on protein utilization in

weanling rats was studied by Viola et al. (47). Cellulose, 55% esterified pectin or 65% esterified pectin, were fed as 10% of the diet. The 55% esterified pectin was also fed as 5% of the diet. All treatments with pectin and cellulose significantly increased the weight of fecal material. Weight gain was significantly less for the pectins fed at the 10% level. Cellulose at the 10% level and pectin fed at the 5% level produced similar weight gains, compared to the control, low fiber-fed rats. The protein efficiency ratio was reduced by the pectins and the cellulose fed at the 10% level, but was increased by the 55% esterified pectin fed as 5% of the diet. Pectin at each level, unlike the cellulose, significantly interfered with protein digestibility. However, the utilization of protein once it had been digested and absorbed was not significantly affected by the 10% pectins or the cellulose and was enhanced slightly by the 5% pectin diet. The 10% pectin appeared to interfere with protein digestion and/or absorption, but did not affect the utilization of the amino acids once they were absorbed (47).

The effect of 0, 10 or 20% microcrystalline cellulose diets on protein utilization was tested by Rao and Sunderavalli (48) in weanling rats. As the cellulose component of the diet increased there were lower urinary nitrogen losses and greater fecal nitrogen losses. Endogenous nitrogen excretion, nitrogen balance and net protein utilization were not significantly affected by the inclusion of cellulose in the diet. The authors suggested that these responses for nitrogen balance and utilization would hold true only when micro-

crystalline cellulose was used as the fiber source.

This experiment was designed to investigate the interaction of protein and fiber in the diet of older animals, as shown by nitrogen balance and the rate of repletion of body weight, serum protein, and liver nitrogen of rats fed fiber free, 10% pectin, or 10% cellulose diets. The apparent relationship of dietary fiber to serum cholesterol and triglycerides was also examined.

## MATERIALS AND METHODS

### Animals

Female Sprague-Dawley retired breeder rats were the experimental animals, ranging from 8 to 10 months of age at the start of the experiment. All animals were fed laboratory chow for four weeks before the experiment began. Animals were randomly assigned from a weight block of seven animals to treatment groups, 13 rats per experimental group. The mean weight of each group was approximately the same. Rats were housed in suspended, wire-bottomed cages with controlled lighting (12 hours dark, 12 hours light) and temperature (72° F). Records of food consumption and body weight were kept for each animal.

### Experimental Design, Diets, and Procedures

A protein depletion, repletion experiment was devised, in which rats were fed a protein deficient diet for 21 days and repleted with a 5% casein diet supplemented with L-methionine for 14 days. Similar time periods have been used in other experiments in protein depletion, repletion studies(49, 50). Diets shown in Table I, were fed ad libitum providing 4 Kcal per gram. The average amount of nitrogen in the protein depletion diets, determined by Kjeldahl analysis, was 0.99 mg per gram of diet. The actual per cent protein in the control, pectin and cellulose repletion diets was 4.3, 4.5 and 4.4, respectively (Appendix, Table II).

Table I  
Experimental Diets g/100g

Ingredient	Control	Pectin <sup>1</sup>	Cellulose <sup>2</sup>
		Depletion	
Cornstarch	82.4	65.7	65.7
Hydrogenated Vegetable Shortening <sup>3</sup>	11.6	18.3	18.3
Minerals <sup>4</sup>	4.0	4.0	4.0
Vitamins <sup>5</sup>	2.0	2.0	2.0
Fiber	0	10.0	10.0
		Repletion	
Cornstarch	77.9	61.0	61.0
Hydrogenated Vegetable Shortening	11.1	18.0	18.0
Minerals	4.0	4.0	4.0
Vitamins	2.0	2.0	2.0
Fiber	0	10.0	10.0
Casein <sup>6</sup>	4.95	4.95	4.95
Methionine <sup>7</sup>	0.05	0.05	0.05

<sup>1</sup>Citrus Pectin, Sunkist Growers, Ontario, CA.

<sup>2</sup>Alphacel Non-nutritive Bulk, ICN Pharmaceuticals, Cleveland, Ohio

<sup>3</sup>Crisco

<sup>4</sup>Salt Mixture Jones Foster, ICN Pharmaceuticals, Cleveland, Ohio

<sup>5</sup>Vitamin Fortification Mixture, ICN Pharmaceuticals, Cleveland, Ohio

<sup>6</sup>Casein, Vitamin Free, ICN Pharmaceuticals, Cleveland, Ohio

<sup>7</sup>L-Methionine, ICN Pharmaceuticals, Cleveland, Ohio

The experimental design by groups is shown in Table II. Groups 1 through 6 were depleted for 21 days on their respective treatments. Groups 1, 2 and 3 were then sacrificed and groups 4, 5 and 6 were repleted for 14 days prior to sacrifice.

Animals were anesthetized with chloroform and a blood sample taken by heart puncture. The animals were then sacrificed with more anesthesia. The blood was allowed to stand at least 45 minutes on ice, centrifuged and serum aliquots frozen for future analyses for triglycerides, cholesterol and total protein. Animals were not fasted before sacrificing to avoid fluctuations in protein values. Livers were excised immediately after death, rinsed in saline, patted dry with wipes, weighed and frozen. They were later freeze-dried, ground with mortar and pestle and aliquots analyzed in duplicate for protein content.

During the last seven days of the depletion and repletion periods, groups 4, 5 and 6 were housed in metabolic cage units designed to trap and separate urine and fecal excreta for each animal. Feed cups were accessible through a tunnel to prevent excessive feed contamination by excreta. Daily collections of urine and feces were made for five days (days 16-20 of the depletion period and days 9-13 of the repletion period). Excretion product composites were frozen immediately after collection for later analysis. Urine was preserved with 0.5 ml 5N HCl for total nitrogen analysis. Feces were weighed, freeze dried, ground with mortar and pestle, and aliquots analyzed in duplicate for nitrogen content.

Table II  
Experimental Design By Groups

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Dietary Treatment	Sacrificed Initially, Baseline Data	21 Days Depletion, Sacrificed	21 Days Depletion, N-Balance, 14 Days Repletion, Sacrificed
Control		1	4
Pectin		2	5
Cellulose		3	6
No Treatment	7		

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### Analytical Procedures

Kjeldahl nitrogen determinations were made on liver and fecal samples according to A.O.A.C. methods (51). The Auto Analyzer II\* was used to determine urinary nitrogen, serum protein, triglycerides and cholesterol. Urinary nitrogen was determined by a modification of industrial method 146/71 A\* in which concentrated sulfuric acid was substituted for the digestion mixture with 30% hydrogen peroxide being introduced after the sample and acid were mixed. Serum triglycerides and cholesterol were extracted from serum using zeolite and 2-propanol and determined colorimetrically as outlined by clinical method 24.\* The method was modified to use 0.3 ml serum per triplicate with the necessary adjustments to maintain the same ratios of the other extraction components. Total serum proteins were determined according to method 14.\*

### Calculations

Net protein utilization was determined by the following formula:

$$NPU = \frac{I - (F - F_0) - (U - U_0)}{I} \times 100$$

where I equalled total nitrogen intake, F and U, fecal and urine nitrogen losses, respectively, during repletion, and  $F_0$  and  $U_0$  equalled total fecal and urine nitrogen losses on the low-nitrogen diet during depletion. Protein efficiency ratio was also calculated for

\*Technicon Instruments Corporation, Tarrytown, New York 10591.

repleted animals with the following formula:

$$\text{PER} = \frac{\text{total weight gain (g)}}{\text{total protein consumed (g)}}$$

### Statistical Analysis

Sacrifice data was obtained from all groups. Groups 4, 5, and 6 supplied nitrogen balance data. Sacrifice data differences between the three depletion groups and the three repletion groups were analyzed using a complete randomized block design. A one-way analysis of variance was used to determine any differences between treatments in the degree of repletion. Scheffe's Multiple Comparison Procedure for Simultaneous Contrasts was used on any variable from the above data sets that showed a significant analysis of variance F value. This procedure provided a way of comparing the means of several treatments and ranking them with respect to the variable in question. Dunnett's Test, a standard comparison test was used to compare sacrifice data of treatment groups 1 through 6 to the baseline group 7.

## RESULTS AND DISCUSSION

### Body Weight

Marked changes occurred in body weight (see Table III) during the depletion and repletion periods. Animals lost approximately one-fifth of their body weight during the three week depletion period, as had been reported by Piedad-Pascual et al. (50) for adult male rats used in research of a similar design. There were no significant differences in percent weight loss during depletion, with the control, pectin, and cellulose treatments averaging 20.8%, 19.8% and 19.2%, respectively. The percent weight gained during repletion was also similar across treatments. The protein deprivation on the low nitrogen diets may have overshadowed any possible treatment effects that could be shown in tissue catabolism during depletion or anabolism during repletion.

### Sacrifice Data

The effects of feeding a low nitrogen diet for 21 days, followed by a protein repletion diet for 14 days, on liver weight, liver nitrogen and serum protein are shown in Table IV. Similar values in these parameters have been reported by Kenney et al. (49) in an experiment with adult male rats. Repleted rats had significantly greater liver wet weight, total liver nitrogen per 100g of body weight and serum protein than depleted animals ( $p < 0.05$ ). Liver wet weights were significantly lower in depleted groups 1, 2 and 3 than for the base-line group 7 ( $p < 0.08$ ). Repleted groups 4, 5 and 6 did not differ

Table III

Effect of pectin and cellulose on body weight  
of aged rats during protein depletion and  
repletion

	Control, 21 Days Depletion	Pectin, 21 Days Depletion	Cellulose, 21 Days Depletion	Control, 21 Days Depletion, 14 Days Repletion	Pectin, 21 Days Depletion, 14 Days Repletion	Cellulose, 21 Days Depletion, 14 Days Repletion
Initial body wt., g	340.8±9.3 <sup>1</sup>	342.7±10.1	342.5±8.9	343.1±8.6	341.5±10.0	336.8±9.0
Depleted body wt., g	264.6±6.2	277.5±7.2	274.7±6.7	276.2±6.9	270.3±8.0	273.8±7.4
% Wt. loss	22.2±1.0	18.8±1.2	19.7±0.8	19.4±0.9	20.7±1.2	18.6±1.1
Repleted body wt., g	n.a.	n.a.	n.a.	304.1±7.8	297.4±8.8	302.1±7.6
% Wt. gain	n.a.	n.a.	n.a.	10.1±0.8	10.1±1.2	10.4±0.9

<sup>1</sup>Mean ± S.E. (This was not the pooled S.E. used in comparison testing for statistical differences.)

n.a. - not applicable to that group

Table IV

Effect of protein depletion and repletion with pectin and cellulose on liver weight, liver nitrogen, and serum protein in aged rats

	Control 21 Days Depletion	Pectin 21 Days Depletion	Cellulose 21 Days Depletion	Control 21 Days Depletion 14 Days Repletion	Pectin 21 Days Depletion 14 Days Repletion	Cellulose 21 Days Depletion 14 Days Repletion	Baseline, No Treatment
Liver Wt. wet, g <sup>2,4</sup>	8.4±0.3	8.3±0.2	8.1±0.3	9.9±0.4	10.0±0.4	9.8±0.4	11.0±0.4
Liver Wt., dry, g	2.8±0.2	2.7±0.1	2.5±0.1	3.5±0.3	3.6±0.3	3.6±0.3	3.3±0.1
mg N/g liver, wet <sup>3</sup>	22.3±0.7	24.2±0.5	24.5±0.7	24.9±0.8	24.8±0.6	24.7±0.6	31.3±0.6
mg N/g liver, dry <sup>3</sup>	71.2±3.6	75.5±2.4	79.2±3.5	73.4±4.8	71.8±3.9	71.5±3.9	106.2±1.2
Total liver N mg/100g Body Wt. <sup>3,4</sup>	72.3±1.7	73.0±1.5	71.1±1.0	80.4±1.8	83.3±1.4	80.3±2.1	100.3±2.2
Serum Protein <sup>2,4</sup> g/100 ml	5.42±0.10	5.02±0.08	5.22±0.03	6.22±0.14	5.69±0.16	6.19±0.13	6.19±0.11

<sup>1</sup>Mean ± S.E. (This was not the pooled S.E. used in comparison testing for significant differences.)

<sup>2</sup>Baseline Value significantly higher than depletion groups, but not different from repletion groups (P<0.08).

<sup>3</sup>Baseline Value significantly higher than all other groups (P<0.08).

<sup>4</sup>Repleted values were significantly greater than depleted values (P<0.05).

significantly from the baseline group in this parameter. The average dry weight of the liver was not significantly different for any treatment. All treatment groups had significantly lower liver nitrogen concentration, on a wet and dry basis, and significantly lower total liver nitrogen per 100g body weight than the baseline group.

Although the average wet weights of the livers of repleted animals were not different from group 7, these animals had less protein in their liver, as shown by the reduced nitrogen concentration. This appeared to be caused by increased fat content in the liver. Unpublished data from this laboratory indicated that fat accumulated in the livers of animals while on depletion diets, as expected in protein malnutrition. Repleted animals, although having equal liver weights, had more fat in their livers than rats sacrificed for baseline data, thus causing reduced nitrogen concentration. There was a strong negative correlation (-0.71) between liver wet weight and milligrams of nitrogen per gram of liver. Fiber-fed rats had greater liver fat than the control group. This could be due in part, to the increased fat component of the fiber-supplemented diets. To maintain isocaloric diets it was necessary to have more fat in the fiber diets than in the control, no fiber diets. It was felt that similar energy concentrations across treatments were necessary, since protein utilization was of primary concern.

The failure of liver nitrogen values to replete to baseline values in 14 days suggested that a longer repletion period was needed. A two week repletion period, however, had been used by Piedad-Pascual

et al. (50) with adult male rats, fed 5 or 10% protein diets. Kenney et al. (49) reported that diets with 18% protein produced normal hepatic nitrogen values after two weeks, but 9% protein diets required more time, when adult male rats were used. The lower protein content of the experimental diets, 5%, in this research, was probably partially responsible for the slower repletion of liver nitrogen. The older age of the animals used for this study might also have increased the time required for complete repletion.

Serum protein values in groups 4, 5 and 6 did replete to baseline values, and were significantly higher than serum protein values from the depleted groups ( $p < 0.08$ ). There was a trend toward lower serum protein levels in pectin-fed rats than in control, no fiber-fed rats. The cellulose-fed animals had intermediate serum protein values. This relationship was found in comparisons between depletion groups and repletion groups. Pectin may have slightly interfered with dietary protein digestion or absorption in the repletion period, as has been reported by Viola et al. (47) and with reabsorption of endogenous protein in the gut during depletion, thus causing the lower serum protein levels. These possibilities will be discussed more fully in terms of fecal and urinary nitrogen losses.

#### Serum Cholesterol and Triglycerides

Serum cholesterol and triglycerides across treatment groups as shown in Table V showed no significant differences when compared to the baseline group. Repleted values were significantly higher than

depleted values ( $p < 0.05$ ), but there were no fiber effects. Previous reports however, have found lower serum cholesterol with pectin feeding (24-29) and with high levels of cellulose feeding (32-34). The failure of pectin to lower serum cholesterol might have been caused by the higher fat content of the fiber diet, 18%, than the control diet 11%. As previously discussed, the fiber diets required a higher fat content to keep all treatment diets isocaloric. Tsai et al. (29) also found that a 0.5% cholesterol-supplemented diet with 7% pectin and 15% fat failed to show the hypocholesterolemic effect of the same diet with only 10% fat in young rats. These results indicated the complex dietary interactions that affect serum cholesterol levels. The higher fat component of the cellulose diet similarly might also have prevented any hypocholesterolemic effect of that fiber source. However, experiments showing beneficial effects on serum cholesterol by cellulose have used twice as much cellulose in the diet as was used in this experiment (30-32). Lowering of serum cholesterol by cellulose might be dependent on high levels of ingestion of this fiber. The failure of the cellulose diet to alter serum cholesterol levels agreed with previously reported experiments in which diets contained 16 g of cellulose per day when fed to humans (2) and 7% cellulose when fed to rats (29). Animals were not fasted when serum cholesterol and triglycerides samples were taken. This may have affected cholesterol values slightly and probably contributed strongly to the variability of triglycerides values. The wide range of serum triglycerides within each treatment group, reflecting different feeding patterns of

Table V

Effect of pectin and cellulose on serum cholesterol and triglycerides of aged rats after protein depletion, and repletion

	Control, 21 Days Depletion	Pectin, 21 Days Depletion	Cellulose, 21 Days Depletion	Control, 21 Days Depletion, 14 Days Repletion	Pectin, 21 Days Depletion, 14 Days Repletion	Cellulose 21 Days Depletion, 14 Days Repletion	Baseline, No Treatment
Serum Cholesterol <sup>2</sup> mg/100ml	53.6±3.2 <sup>1</sup>	65.4±4.2	66.0±4.0	72.1±3.2	78.1±3.3	75.5±2.6	64.9±2.8
Serum Triglycerides mg/100ml	56.3±6.6	81.8±7.5	75.1±9.1	116.0±13.4	73.5±9.0	82.3±11.0	89.5±8.3

<sup>1</sup> Mean ± S.E. (This was not the pooled S.E. used in comparison testing for statistical differences.)

<sup>2</sup> Repleted values were significantly greater than depleted values (P<0.05). Baseline group was not significantly different from other groups.

the animals, masked any real differences that may have been present.

#### Protein Utilization and Nitrogen Balance

The depletion diets contained a small amount of nitrogen (Appendix, Table II). Thus the nitrogen content of fecal and urine composites were not true metabolic fecal nitrogen or endogenous urinary nitrogen values. They were similar however, to metabolic fecal nitrogen and endogenous urinary nitrogen losses reported by other experimenters (48, 52) when allowances were made for differences in animal age, size and food consumption. Therefore, they were used to represent  $F_o$  and  $U_o$  in calculation of net protein utilization.

Nitrogen losses and protein utilization of groups 4, 5 and 6, Table VI, showed significant treatment effects. Fiber diets caused greater fecal nitrogen losses, in agreement with research previously reported, (36, 39, 42, 43, 45, 46, 48). The weight of fecal material in repletion (see Appendix) was highly correlated with repleted fecal nitrogen losses (0.77), which increased with the fiber diets. The fiber increased the bulk of material passing through the gut, probably promoted more rapid transit, which may have reduced protein digestion and/or absorption, causing increased fecal nitrogen. Other experimenters have used this hypothesis to explain increased fecal nitrogen losses or lowered protein digestibility with high fiber diets (38, 43, 46, 47). The pectin-fed animals had significantly greater fecal nitrogen losses during depletion and repletion periods than the cellulose-fed or control animals, yet there was no difference

in protein consumption (see Appendix). Cellulose-fed rats had slightly greater fecal nitrogen than controls, but this difference was not significant. The pectin apparently interfered with protein absorption or reabsorption more than cellulose. This interaction between pectin and protein could be partially responsible for the trend toward lower serum protein values in the pectin-fed group. Decreased apparent protein digestibility has been reported previously when 10% or 5% pectin diets were fed to weanling rats (47).

Fiber-fed animals had less urinary nitrogen losses than control animals, with significant differences shown in Table VI. Increased fecal nitrogen losses and decreased urinary nitrogen losses with fiber have been reported elsewhere (48). Rats on pectin treatment lost significantly less nitrogen in the urine during depletion and repletion periods than the control animals ( $p < 0.05$ ). However, the total nitrogen losses in each period for all three groups were similar. Despite increased fecal nitrogen losses, indicating less protein absorption with pectin in the diet, the weight gain of animals on different treatments was the same, suggesting a more efficient use of the protein that was absorbed. The decreased urinary nitrogen excretion of the pectin-fed rats during repletion indicated that the animals were conserving nitrogen. However, the requirement for protein was met in each treatment, and the obligatory urinary nitrogen losses measured during the depletion period was exceeded by all groups during repletion. Viola et al. (47) also found that protein absorbed from a pectin diet had equal or greater utilization once absorbed.

Table VI

Effect of pectin and cellulose on nitrogen losses,  
NPU, and PER in protein repleted aged rats

21 Days Depleted 14 Days Repleted Groups	Control	Pectin	Cellulose
Urine N loss, mg/Day, Depletion	30.8±0.4 <sup>a1,2</sup>	24.1±0.3 <sup>b</sup>	24.1±0.4 <sup>b</sup>
Fecal N loss, mg/Day, Depletion	13.3±0.1 <sup>a</sup>	20.1±0.2 <sup>b</sup>	15.3±0.1 <sup>a</sup>
Total N loss, mg/Day, Depletion	44.1	44.2	39.4
Urine N loss, mg/Day, Repletion	43.1±0.4 <sup>a</sup>	27.6±0.2 <sup>b</sup>	34.3±0.6 <sup>ab</sup>
Fecal N loss, mg/Day, Repletion	16.6±0.2 <sup>a</sup>	32.7±0.3 <sup>b</sup>	22.2±0.2 <sup>a</sup>
Total N excretion, mg/Day, Repletion	59.7	60.3	57.5
NPU	86.0±0.02 <sup>a</sup>	86.0±0.02 <sup>a</sup>	86.0±0.01 <sup>a</sup>
PER	2.53±0.12 <sup>a</sup>	2.27±0.12 <sup>a</sup>	2.33±0.12 <sup>a</sup>

<sup>1</sup>Mean ± S.E. (This was not the pooled S.E. used in comparison testing for significant differences.)

<sup>2</sup>Values in same line not sharing a common superscript letter are significantly different (P<0.05).

Net protein utilization was calculated using the nitrogen balance data. Average NPU for the three groups was the same showing that pectin and cellulose fed as 10% of the diet did not demonstrate a significant effect on protein utilization in this experiment. Repletion diets with less protein may have showed a significant effect on NPU, particularly with pectin feeding. The pectin-fed rats, despite increased fecal nitrogen losses showing less protein absorption, were still able to absorb enough protein to meet their needs. A lower, more critical protein level in the diet may have prevented these animals from meeting their requirement due to decreased absorption. Slightly lower (80%) NPU values have been reported with a 20% cellulose, 10% protein diet (48) than were found in this study. It would be expected that a diet of higher protein content would be utilized less efficiently than a diet that was more restrictive in protein.

Protein Efficiency Ratio (PER), commonly used to measure protein utilization in young, growing animals was also used in this experiment. Since the animals were depleted of protein for three weeks, it was assumed that weight gained during repletion reflected growth of new tissue, and could therefore be related to protein consumption. The average values of the pectin and cellulose groups were less than that of the control group, but the differences were not significant. This finding does not support the work of Viola et al. (47) who found that 65% esterified pectin fed as 10% of the diet had a deleterious effect on PER in weanling rats. Their research used a 10% casein

diet, with fiber sources substituted for equal measures of starch. Diets were, therefore, not isocaloric. The average PER for the control group in this experiment with older animals was lower than that reported by Viola et al. (47), while PER values for fiber-fed rats were similar to those reported by these authors. The difference in dietary treatment, the age of the experimental animals, the stress of a depletion, repletion study and the adaptation of the PER for use with older animals could explain this discrepancy.

Mean values of other data collected in this experiment can be found in the Appendix.

## SUMMARY AND CONCLUSIONS

An experiment was conducted to determine the effect of the purified fibers pectin and cellulose on protein utilization in aged animals. Female Sprague-Dawley rats, retired breeders, were used and were 8 to 10 months old at the start of the experiment. A protein depletion, repletion study was designed in which animals were fed a low nitrogen diet for 21 days, followed by a 5% casein diet for 14 days. Effects of the regime on weight gain, hepatic nitrogen and liver weight, serum protein and nitrogen balance were examined. Serum cholesterol and triglycerides were also determined.

There were increased fecal nitrogen losses with the pectin diet compared to the control and cellulose diets ( $p < 0.05$ ). The pectin-fed animals also showed a trend toward lower serum protein levels ( $p < 0.10$ ) which could be an effect of reduced protein digestibility or absorption with pectin feeding as indicated by greater fecal nitrogen losses. However measurements of net protein utilization and protein efficiency ratio failed to show any significant differences between treatments in protein utilization. Diets with less protein may have demonstrated an effect of fiber on these parameters. The results of this experiment indicate a need for further study of the pectin-protein interaction in the gut.

The 14 day repletion period appeared not to be adequate for the animals used in this experiment. Although serum protein levels of the repleted group were not significantly different from those of

the baseline group, the hepatic nitrogen concentrations of these groups were significantly below those of the initially sacrificed rats. Livers of depleted and repleted animals showed evidence of fat accumulation which may have hindered the restoration of previous nitrogen levels in the liver. Comparisons with research reported in the literature revealed that the old animals used in this experiment may have been less capable of recovery from a depletion, repletion regime and therefore required a longer time period for complete repletion.

Contrary to literature reports, the hypocholesterolemic effect of dietary fiber was not found in this study. The higher fat component of the fiber diets compared to the control diet apparently induced a hypercholesterolemic effect that the fiber did not overcome. No conclusions could be reached from triglyceride data since these values were highly variable and from non-fasting animals.

Moderate increases in dietary fiber consumption would be unlikely to adversely effect protein nutriture in humans if an individual was consuming an adequate protein diet. However, care should be exercised in recommending high levels of fiber, particularly if protein status is marginal, since fiber feeding does increase fecal nitrogen excretion. Human nitrogen balance experiments would be helpful in clarifying the effect of fiber on protein utilization in man. The interaction of fiber with other essential nutrients in the human diet such as minerals, should be examined before drastic changes in fiber consumption are recommended by nutritionists.

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APPENDIX

Table I

Fecal weights and food consumption  
of protein repleted older adult rats

21 Days Depleted 14 Days Repleted Groups	Control	Pectin	Cellulose
Depleted Fecal Wt., wet g	2.2±0.1 <sup>1,2a</sup>	3.6±0.3 <sup>a</sup>	6.8±0.4 <sup>b</sup>
Depleted Fecal Wt., dry g	1.8±0.1 <sup>a</sup>	2.9±0.2 <sup>a</sup>	6.0±0.4 <sup>b</sup>
Repleted Fecal Wt., wet g	3.2±0.2 <sup>a</sup>	6.1±0.7 <sup>b</sup>	10.8±0.6 <sup>c</sup>
Repleted Fecal Wt., dry g	2.7±0.2 <sup>a</sup>	4.8±0.3 <sup>b</sup>	9.6±0.6 <sup>c</sup>
Total Food Consumed	262.6±10.8 <sup>a</sup>	261.4±12.4 <sup>a</sup>	278.4±11.1 <sup>a</sup>
Total g protein consumed per 100 g body wt.	3.7±0.1 <sup>a</sup>	4.0±0.2 <sup>a</sup>	4.1±0.2 <sup>a</sup>

<sup>1</sup>Mean ± S.E. (This was not the pooled S.E. used in comparison testing for significant differences.)

<sup>2</sup>Values in same line not sharing a common superscript letter are significantly different (P<0.05).

Table II

Nitrogen content of experimental  
diets as determined by Kjeldahl analyses

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Diet	Nitrogen mg/g
Control Depletion	0.86
Pectin Depletion	1.03
Cellulose Depletion	1.06
Control Repletion	6.84
Pectin Repletion	7.16
Cellulose Repletion	7.04

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THE EFFECT OF PECTIN AND CELLULOSE  
ON PROTEIN UTILIZATION  
IN OLDER ADULT RATS

by

JANE GARRETTE SCHWEITZER

(ABSTRACT)

An experiment was conducted to determine the effect of a 10% pectin or 10% cellulose diet on protein utilization in older adult rats. Animals were fed a protein free diet for 21 days, followed by a 5% casein diet supplemented with L-methionine for 14 days. Urine and feces were collected for five days at the end of depletion and repletion periods and analyzed for nitrogen content. Blood samples were taken by heart puncture for serum protein, triglycerides and cholesterol analyses. Livers were excised and analyzed for nitrogen content.

There were no differences in the degree of repletion between treatments as measured by body weight, total liver nitrogen and liver nitrogen concentration. There was a trend toward lower serum protein in the pectin-fed rats and fecal nitrogen losses were significantly greater in this group than in control or cellulose-fed rats. Urine nitrogen losses were significantly lowered with pectin feeding. Calculations of net protein utilization and protein efficiency ratio showed no significant differences between treatments. The increased fecal nitrogen loss of animals with pectin feeding indicated less protein absorption in the

gut, but animals were still able to meet their requirement as shown by the degree of repletion and urinary nitrogen losses. A difference in protein utilization may have been found if repletion diets were lower in protein. Further study of the pectin, protein interaction in the gut was suggested.